Texture changes and myosin oxidation during ripening of salted herring

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Abstract

During the production of old fashioned salted herring, fish are stored for up to 12 months in brine. The brine contains a high level of salt and haemoglobin, which is a strong prooxidant and is believed to induce protein cross-linking. The changes in texture during ripening were investigated using the texture profile analyzer (TPA). In addition, the rate of free radicals formation was evaluated using electron spin resonance spectroscopy (ESR). Myosin degradation and cross-linking were determined using SDS-PAGE and western blotting, respectively. Samples were analyzed after 2, 85, 151 and 371 days of ripening. The TPA revealed an increase in hardness at 2 days of ripening and a significant decrease at 371 days. The increase in hardness could be explained by myosin cross-linking and formation of aggregates. Degradation of myosin aggregates corresponded to the decrease in hardness observed at 371 days of ripening. In conclusion, oxidative reactions inducing myosin cross-linking is likely to be responsible for the textural changes in salted herring. It is yet to be confirmed if haemoglobin is the main actor in these oxidative reactions.

Introduction

In Northern Europe salted herring is a popular fish product, and some of its production is today still based on the traditional process, where whole intact herrings are placed in barrels with salt overnight, and a characteristic blood brine is formed. Thereafter, the barrels are filled with saturated brine and stored for up to 12 months at chilling temperature. During ripening many biochemical reactions take place and the herring develop the characteristic taste and texture of salted fish. Texture changes during ripening are normally determined using highly trained and qualified staff compressing the herring and giving a subjective evaluation of its texture. Only a limited number of studies have used instrumental measurement of texture changes during ripening of salted herrings, which might be due to the complex structure, composition and rheology of fish muscle, but also to the complexity of the changes taking place during ripening. Even though the fish develop a rather firm texture, which is very characteristic for salted fish, substantial proteolytic breakdown is occurring in the fish muscle during ripening (Olsen & Skåra, 1997). Most of the research on understanding the ripening process, has investigated the role of proteolytic enzymes and their relation to protein breakdown (Nielsen & Børresen, 1997). However, until now the correlation between protein modifications and texture has not been investigated and very little research has been performed on the relationship between brine composition, protein oxidation, and texture changes during ripening (Andersen et al., 2007). In the current study, salted herring were produced according to the traditional recipes and the relationship between protein modifications and texture of salted herring were investigated.

Material and methods

Fresh caught whole intact herrings were dry salted (12% w:w) in barrels overnight, and a blood brine containing proteins, salt, and soluble matter from the herrings was formed. The next day the barrels were topped up with saturated brine, closed and stored at 2 °C for up to approximately 1 year. Herring samples were taken out after 2, 32, 85, 151, and 371 days and biochemical analysis were performed. Lipid oxidation was measured using peroxide values (Shantha & Decker 1994). Protein oxidation was measured using measurement of protein carbonyl groups (Levine et. al., 1994). SDS-PAGE and western blotting against myosin was performed using an anti-myosin antibody (1:10.000) and developed by chemiluminescence. ESR measurement was performed using a method previously described by Carlsen et al. (2001). In addition changes in texture were measured using a texture profile analyzer (TPA).

Results and discussions

Figure 1 shows that no significant lipid oxidation occurred during ripening. In contrast, protein carbonyl groups increased significantly and rapidly indicating significant protein oxidation. A maximum for

protein carbonyl groups was reached after 151 days ripening. Thereafter, a decrease in protein carbonyl groups was observed probably due to the decrease in TCA precipitable protein.

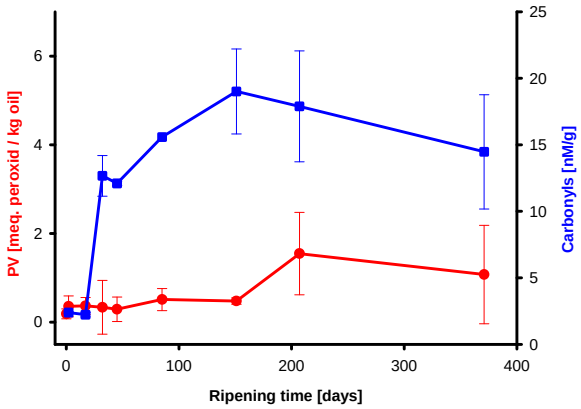

Figure 1: Formation of protein carbonyl groups (blue line) and lipid peroxide (red line) in herring as a function of ripening time. days.

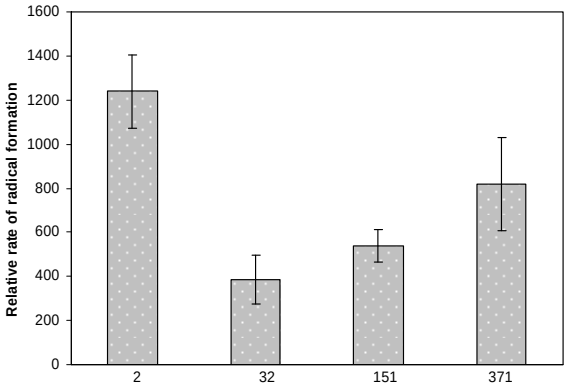

Figure 2: Relative rate of free radical formation in herring homogenate using PBN as spin trap after 2, 32, 151 and 375 of ripening.

Figure 2 revealed that at 2 days of ripening the herring muscle had a strong tendency to generate free radicals. However, the ability to form radicals was significantly lower at 32 days of ripening when compared to day 2. Thereafter, an increase in free radical formation was observed with increasing ripening time (Figure 2). This increase in free radical formation may be explained by the strong peroxidase activity detected in the brine and persisting with ripening time as described earlier (Andersen et al., 2007). From TPA analysis (Figure 3) it appeared that hardness of herring muscle was significantly higher after 2 days of ripening when compared to fresh fish (raw).

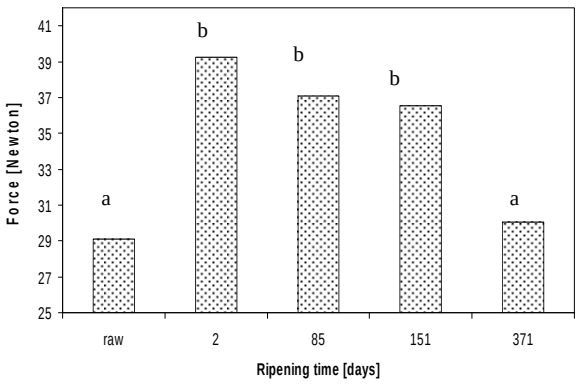

Figure 3: Hardness of herring fillets as a function of ripening time (raw, 2, 85, 151 and 371 days of ripening) measured using the Texture Profile Analyzer. [a,b] Different letters of superscripts indicate significant differences ($p<.05$).

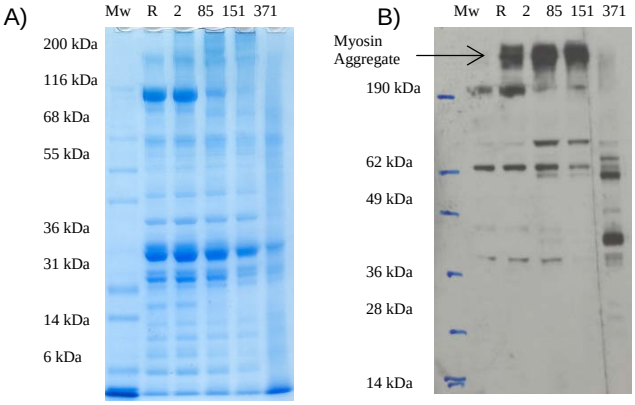

Figure 4: A) SDS-PAGE of herring homogenate and B) Western blotting using an antibody against myosin. Mw-Standard; R: Raw herring, and herring after 2, 85, 151, and 371 days of ripening.

The texture remained firm for up to 151 days of ripening despite the extensive breakdown of actin and myosin as seen in Figure 4A. At 151 days, myosin was almost completely degraded while the fish texture remained firm. The increase in hardness correlated with the formation of myosin aggregate as observed in figure 4B. Figure 4B shows that at 371 days of ripening the myosin aggregates are degraded, and this correspond to the extensive proteolysis (Figure 4A) and softening of the fish muscle observed in Figure 3.

The results all together indicate a significant level of protein oxidation in salted fish during ripening as revealed by increasing carbonyl groups, increasing rate of free radical formation and by the presence of a significant amount of cross-linked myosin. The characteristic texture of salted fish is therefore likely to be caused by the combination of both protein oxidation and proteolytic breakdown.

Conclusions

Despite significant proteolytic breakdown of muscle protein during ripening we postulate that free radicals induce protein oxidation and myosin cross-linking, which is likely to contribute to the increased in hardness of salted herring. More investigation is needed in order to elucidate if protein oxidation not only results in protein cross-linking but also contributes to protein breakdown.

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